THE USE OF MICROSYSTEMS FOR THE EVALUATION OF THE EFFECT OF TOXICANTS *)

KEES KERSTING

(Research Institute for Nature Management,
Kasteel Broekhuizen, Leersum, The Netherlands)

INTRODUCTION

For the determination of the effects of environmental factors the need of experimentation with complex systems is obvious. Experimentation with single organisms fails to find effects inherent in the complexity of nature. On the other hand the interpretation of the results of field experiments is very difficult. An approach that has some of the advantages of both is the use of laboratory scale ecosystems. These systems can be kept under more or less constant conditions and can be manipulated, which makes replicate experiments possible. If those systems can be maintained for longer periods, they must have some of the intrinsic properties of ecosystems. Experimentation with these systems might be very useful for the evaluation of the effects of environmental factors.

Many attempts have been made to establish microecosystems. Reviews are given by COOKE (1971) and TAUB (1974). In most cases the properties of these systems and especially metabolism and succession have been studied. Few papers are published in which the microecosystem approach is used to test environmental factors. One of the best examples is given by COOPER and COPELAND (1973). They studied the effect of the quantity and quality of freshwater on estuarine microecosystems. In those systems and in the systems of TAUB (1972), SÖDERGREN (1973) and UHLMANN (1971) there is a continuous supply of new medium and therefore no problems arise due to insufficient recycling of nutrients. However, recycling of nutrients is one of the basic properties in many ecosystems. The effect of any factor on the mineralizing subsystem is not detected in continuous or semi-continuous flow experiments. In material closed microecosystems like the ones of BEYERS (1963), TAUB (1969), COOPER (1973), ABBOTT (1966) and COOKE (1967) there is no input of nutrients. However, TAUB (1969) found that the nutrient regeneration was insufficient and she had to add new culture medium during the experiment. In the experiments of BEYERS, COOPER and ABBOTT the sediments that were added to the microcosms might have served as a nutrient supply for the duration of the experiments.

Another problem with the one compartment systems is pointed out by NIXON (1969). Due to the small size of the systems overgrazing by the herbivores can be a problem. NIXON (1969) solved this problem by placing a small cheese cloth covered glass tube in his microecosystem. This glass tube is free from the grazing pressure of the herbivore and can serve as a continuous seed for the algal population. Another method to avoid this problem of overgrazing is the use of multi-compartment systems connected by an unidirectional flow of water. If the output of the last compartment is used as input for the first compartment, recycling of nutrients is possible. This approach has been used for some years in

*) RIN communication No. 148.

the Limnological Laboratory of the University of Amsterdam. Two sizes of microecosystems have been developed. A system with a total volume of 175 liter is set up for a study of the material and energy budgets. The big size makes it possible to sample without disturbing the system too much. A second system with a volume of about 6.5 to 10 liter is used as a bioassay system. The prototypes of both systems have been functioning for two years without the need of reintroducing organisms into the system. A paper which deals with the principles and some results of these prototypes is in preparation (Ringelberg and Kersting). In the present paper some results are presented of the testing of the effect of diuron on the small size systems.

Diuron (N-3,4-dichlorophenyl-N', N'-dimethylurea) is a very powerful herbicide, that also has algicidal effect. It was the theme of a special Symposium of the Netherlands Hydrobiological Society in 1969 (Hydrobiol.Ver., 1970). The papers presented at the symposium indicate that diuron is very effective at low concentrations (0.1 - 0.4 ppm), had no negative effects in field experiments on fish or zooplankton, but is persistant, is accumulated by fish and evertebrates, and is toxic to fish and zooplankton at higher concentrations. The toxicity data given at the Symposium and those of COPE (1966) indicate that the effects become evident at concentrations above 1 ppm. Except for RINGELBERG's (1970) experiments these were toxicity tests for the survival of individual animals. However, for the determination of eventual harmful effects one has to know the effect on the survival of populations. It was this information on the survival of a population that was the aim of the experiments with microecosystems.

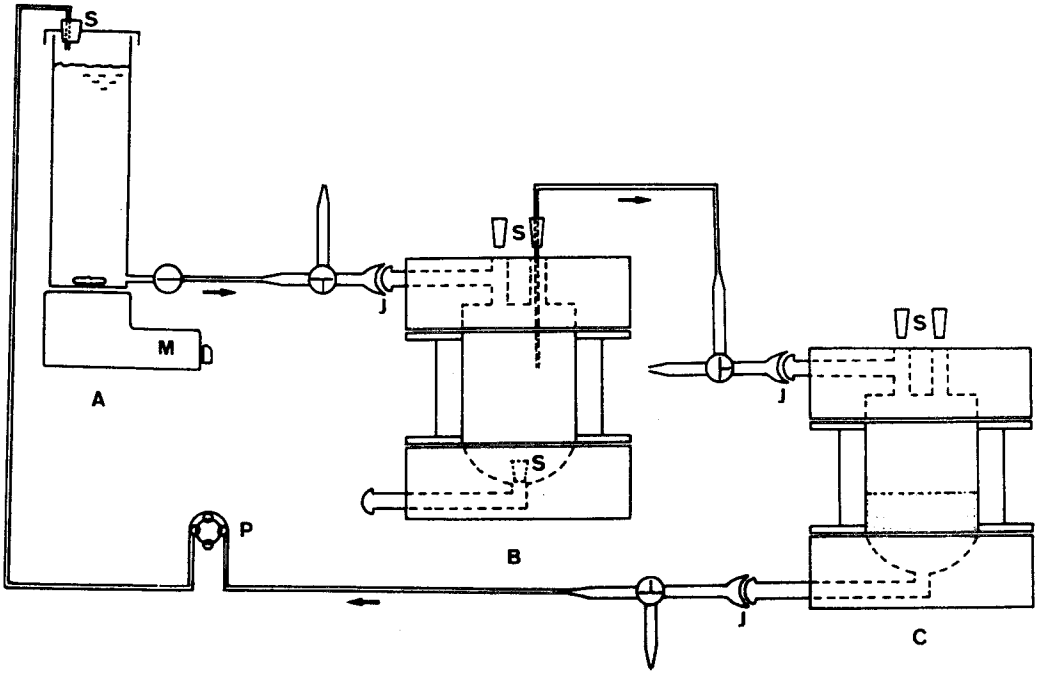

Fig. 1. Experimental system. For the discription see text.
S = rubber stopper, M = magnetic stirrer, P = peristaltic pump,
J = ground glass ball joints.

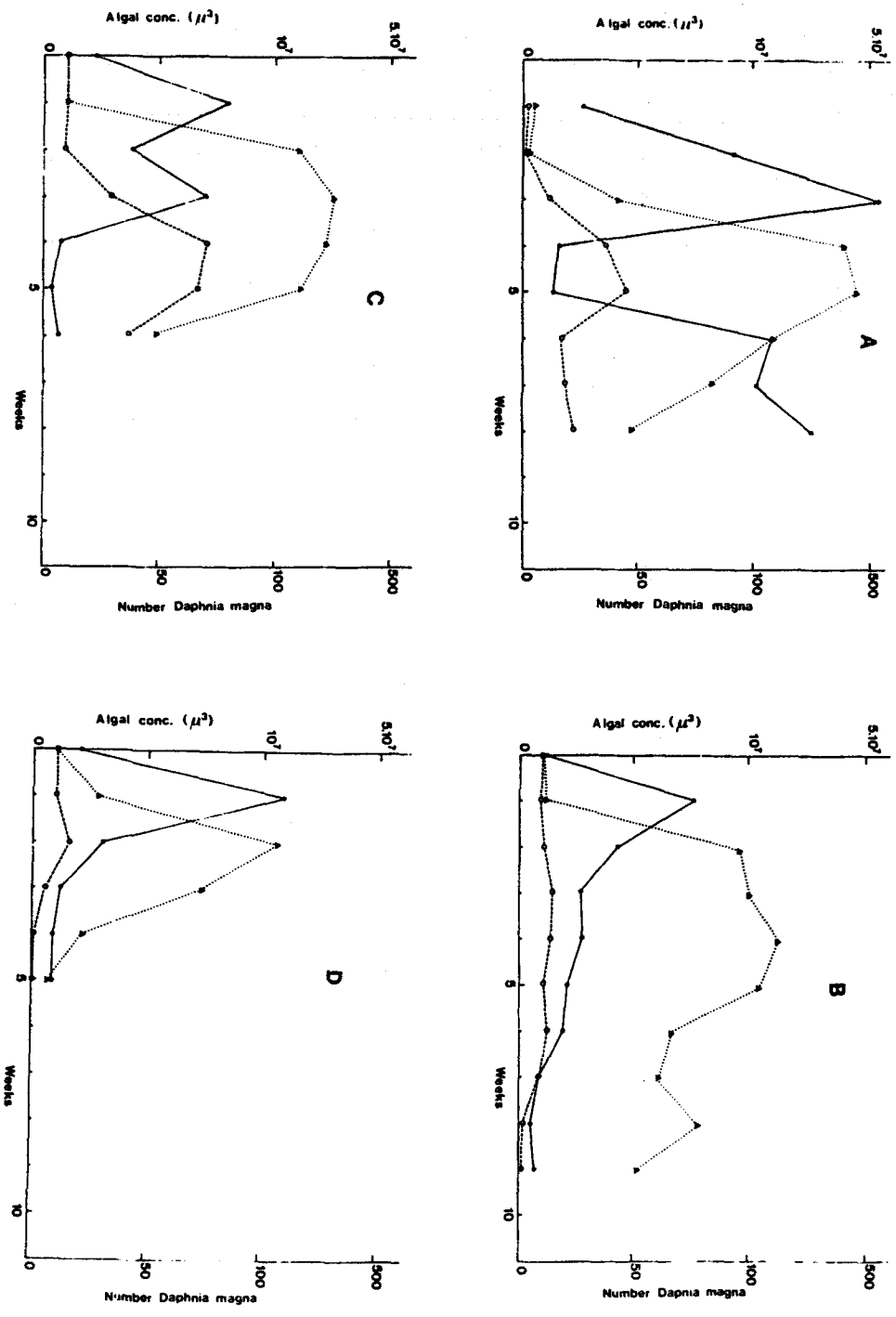

Fig. 2. Algal concentration and number of *Daphnia magna* in the micro-ecosystem plotted against time.
A and C are control experiments.
In B an D diuron was added to compartment B one week after the start of the experiments.
——•—— Algal concentration in compartment A
·····•····· total number of *Daphnia magna*
·····○····· number of adult *Daphnia magna*
Ordinates are linear from zero to 10^7 resp. 100 and logarithmic above these values.

MATERIALS AND METHODS

The experimental set-up is shown in Fig. 1. Compartment A is a plexiglass cylinder with a volume of about 7 liter, which is filled with 6 liter algal suspension at the start of the experiments. At the bottom the compartment is connected via a stopcock to the second compartment in which *Daphnia magna* are placed. This plexiglass compartment consists of three parts that can be screwed together air and water tight. Through a glass capillary the second compartment is connected to the identical compartment C. Between the bottom and middle part of compartment C a 0.2 μ membrane filter is fixed and on top of this filter a layer of sand is placed as a substratum for bacteria. The sand had been heated at 600°C for some hours to destruct all organic material. Through the bottom of compartment C the water is sunctioned away by a peristaltic pump and pumped into the first compartment. As the compartments B and C are completely closed, this single pump is sufficient to drive a continuous flow from compartment A to compartment B, through the glass capillary to compartment C and through the sand/membrane filter back to compartment A. The connections between the compartments are silicon tubings and the pump has a tygon tubing. Compartment A is stirred by a magnetic stirrer. The volume of the compartments B and C is about 175 ml.

Compartment A is illuminated by a circular fluorescent lamp with a 10 hours dark 14 hours light cycle. Compartment B is shielded from this lamp and therefore has an illumination of a fairly low intensity. Compartment C is kept completely dark. The system is placed in a constant temperature room with a temperature of 18°C. The flow through the system is between 600 and 700 ml per day.

The experiments were started by filling the whole system with a very dilute suspension of 10 ml *Chlorella vulgaris* culture in 8 liter membrane filtered water from a eutrophic lake. In the second compartment 10 adult female *Daphnia magna* were placed and the whole system was closed and the flow was started. Every week the particle concentration in compartment A was determined with a Coulter Counter (model Z_B with C 1000 Channelyzer) and the daphnids in compartment B were counted. The daphnids were counted separately as adults and non-adults.

Diuron was added one week after the start of the experiments as an 8% granulate (AA Karmex 8% granulate) to compartment B. The concentration was calculated to be 0.2 ppm assuming complete release of the active compound and assuming complete mixing in the total volume of the system.

Daphnia magna and *Chlorella vulgaris* were cultured as described by KERSTING and VAN DER LEEUW-LEEGHWATER (in press).

RESULTS AND DISCUSSION

The results of four experiments are given in Fig. 2. In two cases diuron was added and in two experiments not. The development of the algae is not quite satisfactory. In the control experiments the particle concentration in compartment A decreased dramatically after some weeks. This decrease was caused by wall growth of the algae. In the diuron experiments no wall growth occurred and the decrease of the particle concentration is caused by diuron. As a result of this the particle concentration in the control experiments is not higher than in the diuron experiments.

The effect of diuron on the *Daphnia magna* population in these experiments is rather peculiar. The fecundity is somewhat lower in the presence of diuron, but the juveniles do not grow up to become adults. This will lead to the vanishing of the population. In the diuron experiments the females had eggs and embryos during the whole experimental period. In both diuron experiments the last surviving female at the end of the experiments still had eggs in the brood pouch. On the other hand in the control experiments the females had no eggs or embryos in their brood pouches at the end of the experiments. These observations indicate that the algicidal effect of diuron probably is of minor importance for the vanishing of the *Daphnia* population. INGLE et al. (1937) showed that the food concentration had an effect on the fecundity of *Daphnia* and virtually no eggs are released into the brood pouch at low food concentrations. They also found that the growth and probably the final length too are influenced by the food concentration, but the animals become adult at fairly low concentrations. The longterm observations of the prototype systems as described by Ringelberg and Kersting (in prep.) are in agreement with these results. Another argument against the food shortage explanation of the vanishing of the *Daphnia* population is the fact that the particle concentration is not lower in the diuron experiments than in the control experiments. One therefore has to assume a direct effect of diuron on the *Daphnia magna* population.

The mechanism of this effect is not clear. Diuron probably has an effect on the very young animals and the few animals that do grow up in the diuron experiments are probably released from the brood pouch before or shortly after the introduction of diuron. One mechanism that can play a role is the "predation" by the glass capillary. Only small animals can pass the capillary and the effect of diuron could be an impairment of the avoidance reaction that *Daphnia* have for currents. In future experiments it will be tried to place a 100 μ plankton gauze in front of this capillary to avoid disappearance in this way. It might however be that the succes of the system is coupled to the selective predation on juvenile animals. In this way crowding might be prevented and so the formation of ephipia might be avoided.

There are more technical problems that have to be solved. The problem of the wall growth in compartment A is probably the most important. In these attached algae nutrients are locked up and are not longer available to the free floating algae. As only the "planktonic" algae are transported to compartment B, this wall growth results in food shortage for the *Daphnia*. The use of a vibrating stirrer to keep the algae in homogeneous suspension is now tried out. Another problem is the place of the membrane filter between the bottom and middle parts of compartment C. It is nearly impossible to replace this filter in case of blockage or damage, without disturbing the whole system very much. It is probably much better to place this filter in a standard filter holder between compartment C and the pump while leaving the sand in compartment C. Finally the compartments B and C that were especially built for another research program can be replaced by standard glassware. It will then be possible to set up a series of systems to start an elaborate program of tests.

The interpretation of the diuron effect and the implications for management purpose are yet hard to make. It seems from these results

however, to be allowed to state that diuron has a direct effect on a *Daphnia magna* population. The very low concentration used in the experiments is in the range of the normally applied concentration and has up till now been considered as without effect on animals. The present results make it necessary to revise this idea.

SUMMARY

A recycling multi-compartment algae *Daphnia magna* bacteria microecosystem was used to evaluate the effect of the herbicide diuron. It was found that 0.2 ppm diuron was lethal to the *Daphnia magna* population in this microecosystem. Diuron had an effect on newly born animals that therefore did not grow up to become adults. It was argued that it was a direct effect on the *Daphnia* and not an indirect effect because of food shortage.

ACKNOWLEDGEMENTS

The hospitality of the Dierfysiologisch Instituut of the University of Amsterdam, where the experiments were carried out is greatly appreciated. I am much indebted to Dr. J. Ringelberg for his interest in the subject and for many fruitful discussions.

REFERENCES

ABBOTT, W., 1966. Microcosm studies on estuarine waters. J.Water Poll. Contr.Fed., 38: 258-270.

BEYERS, R.J., 1963. The metabolism of twelve aquatic laboratory microcosms. Ecol.Monogr., 33: 281-306.

COOKE, G.D., 1967. The pattern of autotrophic succession in laboratory microcosms. BioScience, 17: 717-721.

COOKE, G.D., 1967. Aquatic laboratory microsystems and communities. In: J.Cairns Jr., ed., The structure and function of fresh-water microbial communities: 47-85. Research Division Monograph 3, Virginia Polytechnic Institute and State University, Blacksburg.

COOPER, D.C., 1973. Enhancement of net primary productivity by herbivore grazing in aquatic laboratory microcosms. Limnol.Oceanogr., 18: 31-38.

COOPER, D.C. and B.J. COPELAND, 1973. Responses of continuous-series estuarine microecosystems to point-source input variations. Ecol. Monogr. 43: 213-236.

COPE, O.B., 1966. Contamination of the freshwater ecosystems by pesticides. J.appl.Ecol., 3(suppl.): 33-44.

HYDROBIOLOGISCHE VERENIGING, 1970. Toepassing en gevolgen van het herbicide diuron in het water. Meded.Hydrobiol.Ver., 4: 1-47.

INGLE, L., T.R. WOOD and A.M. BANTA, 1937. A study of the longevity, growth, reproduction and heart rate in *Daphnia longispina* as influenced by limitations in quantity of food. J.exp.Zool., 76: 325-352.

KERSTING, K. and C. VAN DER LEEUW-LEEGWATER, 1975. Effect of food concentration on the respiration of *Daphnia magna*. Hydrobiologia (in press).

NIXON, S.W., 1969. A synthetic microcosm. Limnol.Oceanogr., 14: 134-145.

RINGELBERG, J., 1970. De giftigheid van diuron voor *Daphnia magna* (watervlo). Meded.Hydrobiol.Ver., 4: 29-34.
SÖDERGREN, A., 1973. Transport, distribution and degradation of chlorinated hydrocarbon residues in aquatic model ecosystems. Oikos, 24: 30-41.
TAUB, F.B., 1969. A biological model of a freshwater community: A gnotobiotic ecosystem. Limnol.Oceanogr., 14: 136-142.
TAUB, F.B., D.H. McKENZIE, R.C. HUNG, F.E. PALMER, J. MORGAN, J.J. HELLOR, K. HAMEL, 1972. Biological models of freshwater communities. Res.Fish.Coll.Fish.Univ.Wash., 1971 (355): 59-62.
UHLMANN, D., 1971. Influence of dilution, sinking and grazing rate on phytoplankton populations of hyperfertilized ponds and microecosystems. Mitt.Internat.Verein Limnol., 19: 100-124.